Special Topics

Interaction of Soil Moisture Stress and Ambient Ozone on Growth and Yields of Soybeans

H. E. Heggestad, T. J. Gish, E. H. Lee, J. H. Bennett, and L. W. Douglass

Plant pathologist, soil physicist, plant physiologists, and statistical consultant, respectively, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705.

The project was supported in part by the United States Environmental Protection Agency under assistance agreement 12-14-7001-1282 to the U.S. Department of Agriculture.

The authors thank Kenneth L. Tuthill for technical assistance, Stephanie Wilding for computer data analysis, Perry B. Cregan for cultivar recommendations, and Jerry E. Quisenberry (USDA, Lubbock, TX) for suggestions on the presentation of soil water potential data.

Accepted for publication 6 November 1984.

ABSTRACT

Heggestad, H. E., Gish, T. J., Lee, E. H., Bennett, J. H., and Douglass, L. W. 1985. Interaction of soil moisture stress and ambient ozone on growth and yields of soybeans. Phytopathology 75:472-477.

A field experiment was conducted in open-top chambers to determine how interactions of soil moisture stress and exposure to ozone may affect soybean yields. Cultivars Williams and Forrest were grown in 1982 and Williams and Corsoy in 1983. Five levels of O_3—including charcoal-filtered (CF) and nonfiltered (NF) air—and two soil moisture regimes (averaging −0.05 MPa, and about −0.40 MPa at depths of 0.25 and 0.45 m) were employed. This report describes a significant interaction involving soil moisture stress (SMS) and the CF and NF air treatments. Compared to the no-stress treatment, the combination of SMS and ambient O_3 in 2 yr of experiments reduced yields by 25%. Exposure to ambient O_3 without SMS reduced yields 5%. In CF air, SMS lowered yields 4%. Knowledge of this interaction increases the significance of O_3 as an environmental phytotoxicant. Although cultivar differences existed, the soybean plants acclimated to SMS in CF air but not in NF air. These results provide the first experimental evidence that exposure to elevated ambient O_3 may cause plants to lose tolerance to SMS.

Additional key words: air pollution, *Glycine max*, open-top field chambers, plant stress.

Plants grown with soil moisture stress (SMS) are generally believed to be less sensitive to O_3 than are well-watered plants (9,23). The incidence and severity of tobacco weather fleck, a leaf spot caused by O_3 (14), increased when the crop was irrigated (20,29,33). Tobacco weather fleck is primarily acute foliar injury induced by O_3. New leaf injury develops within 24–48 hr after an air pollution episode. Although much is known about the relation of SMS to acute foliar injury induced by a short-term exposure to elevated O_3 concentrations, the effects of SMS and chronic O_3 exposure on seed yields of field-grown plants have not been previously studied.

Consequently, research was needed to determine if supplemental irrigation in field-experiments with open-top chambers as carried out in the National Crop Loss Assessment Network (NCLAN) caused an overestimation of the effects of O_3 on crop yields (10,11). Also, before the NCLAN program supplemental irrigation was used in some open-top chamber experiments to avoid severe moisture stress. For example, we have data since 1972 (12,13). Periods of drought are common during summer months in the eastern United States. Our goal in conducting experiments with SMS was to include a treatment with a moderate level of SMS, enough to reduce soybean yields by 15–20% when compared to a no-SMS treatment. The NCLAN experiment was designed primarily to obtain O_3 dose-response functions for soybeans. This report describes an unexpected, but very important, interaction involving SMS and stress resulting from exposure to ambient O_3.

MATERIALS AND METHODS

Soybean (*Glycine max* (L.) Merr.) cultivars Williams-79 and Forrest were grown in 1982 and Williams-79 and Corsoy-79 in 1983. Williams belongs to the Group III maturity class, Forrest to Group V, and Corsoy to Group II. Corsoy matures about 2 wk earlier, and Forrest 3 wk later, than Williams. Williams has been the most widely grown soybean cultivar in the United States for more than 5 yr. Williams and Forrest are commonly grown in Maryland.

The study was conducted on Cordorus silt loam (a deep, permeable, well-drained soil) at the Beltsville Agricultural Research Center in Maryland. Twelve cylindrical open-top field chambers (3 m diameter × 2.4 m high), each with a blower attached, were used (8); six chambers received charcoal-filtered (CF) air and six nonfiltered (NF) air. The chambers contained single rows (0.91 m apart) of each soybean cultivar. Seeding in 1982 was done on 26 May and in the following year on 9 June. The seedlings were thinned to 5 cm spacing in the row. Recommended management practices for soybeans were followed.

The chambers were in place with the blowers operating from 26 June 1982 and 11 July 1983 until October. The blowers were turned off between 2100 hours and 0500 hours EST each day to allow for dew formation on the plants. Three CF and three NF chamber plots were drip irrigated to prevent SMS and an equal number of them were maintained with a moderate level of SMS at the 0.25–0.45 m control depth. The 2-m center portion of each 3-m row was manually harvested. Seed yields were adjusted to 13% moisture content.

Leaf stomatal conductances were measured with a LI-COR steady-state autoporometer model LI-1600, (LI-COR Inc., Lincoln, NE). Leaf water potentials (ψ_l) were determined with a Scholander pressure chamber (PMS, Corvallis, OR). The stomatal conductance and ψ_l measurements were made on a recently fully expanded leaf, usually on six plants per treatment. Leaf areas and weights were determined at various stages of growth by sampling a terminal leaflet from the fourth trifoliate from the stem tip on each of 10 plants per plot. The leaf area was measured with a LI-COR electronic area meter.

Soil water potentials ψ_s in the no-moisture-stress regime were monitored with tensiometers (Irrometer Co. Inc., Riverside, CA) at 0.25 and 0.45 m depths. The tensiometers are effective only when

---

soil is in the moist range, −0.02 to −0.08 MPa. In the moderate-SMS regime, soil psychrometers (WESCOR PCT-55-15 and model PR-55 Microvolt Meter, Wescor Co., Logan, UT) were employed. Psychrometers can be calibrated to monitor low ψ_s, from −0.15 to −2.0 MPa. The ψ_s values in the well-watered plots were read each morning to determine the need for irrigation to prevent SMS (−0.05 MPa). The soil psychrometers were read at the same time as the tensiometers; however, when the soils in the dry-soil regime were more moist than the targeted ψ_s (−0.40 MPa), readings were not taken the following day. Water was applied as needed by trickle irrigation (AGRIFIM Drip Irrigation, Shemin Nurseries, Inc., Burtonsville, MD). Data on rainfall, temperature, and relative humidity also were collected from May through September.

A TECO model 49 (Thermo Electron Corporation, Hopkinton, MA) and Bendix model 8002 (Bendix Corporation, Lewisburg, WV) were used to monitor O_3. A Dasibi model 1003 PC (Dasibi Environmental Corp., Glendale, CA) was used for calibration.

Rain exclusion. In 1982, clear plastic shields were supported about 0.5 m above the open-top chambers to exclude rainfall from the plots. They were removed as soon as the threat of rain had passed. In 1983, to avoid the handling of rain shelters above the chambers, clear plastic sheeting was placed about 10 cm above the soil to exclude rain from two-thirds of the soil surface in all plots. An area 0.3 m wide in each plant row was left uncovered.

The soil level was raised 5 cm over the center area of four beds (7.6 m × 90 m) and furrows were cut between the beds to aid the removal of surface water. Soil temperatures were monitored with thermocouples beneath the plastic at depths of 0, 5, and 15 cm in a moist and a dry plot. On hot days with ambient air temperatures above 35 C, the soil temperature midway between the plant rows was elevated about 1.0 C at a depth of 15 cm beneath the plastic (0.6 m wide). There was no effect on soil temperatures in the plant row.

Irrigation. All plots contained a drip irrigation system with valves to manually control the irrigation. Control on an individual plot basis was needed to achieve greater uniformity between plots in soil water potentials. In 1982, the lines were laid on either side of each row; 0.15 m from the plants in the dry series and midway between the rows of plants in the moist series. In 1983, because of the plastic rain shelters between the rows, all lines were placed as described for the dry series in 1982. The emitters were 50 cm apart in the lines. By alternating emitter placement on opposite sides of

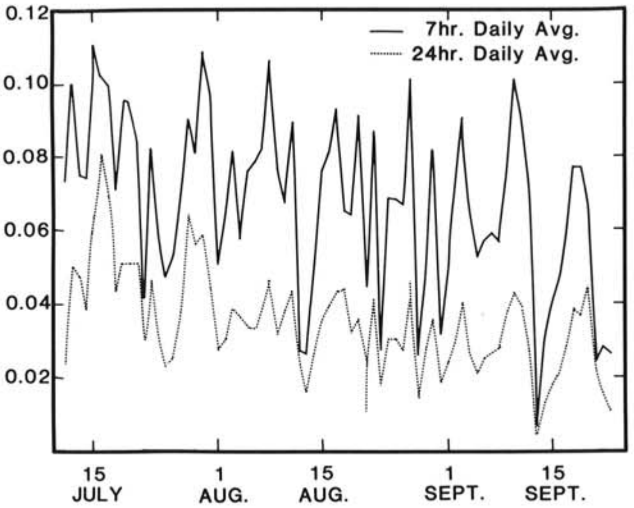

Fig. 1. Daily ambient 7-hr (0900–1600 hours EST) and 24-hr O_3 concentrations from 11 July to 23 September 1983, measured at a height of 3 m adjacent to the soybean field experiment site.

TABLE 1. Total water received on charcoal-filtered (CF) and nonfiltered (NF) plots expressed as a percentage of normal rainfall for July through September (30.6 cm) and May through September (48.8 cm)[a]

O_3 treatment in chambers	1982 Moist (%)	1982 Dry (%)	1983 Moist (%)	1983 Dry (%)
July–September				
CF	190	53	162	39
NF	191	65	164	33
May–September[b]				
CF	163	77	160	83
NF	164	84	161	79

[a] Values shown take into account rainfall, water excluded by rain shields, and that provided by irrigation.

[b] Rainfall was 7.1 cm less for May and June in 1982 than in 1983; since soybean plants are deep rooted and can utilize to some extent stored ground water, the total amounts available from May to September are considered important.

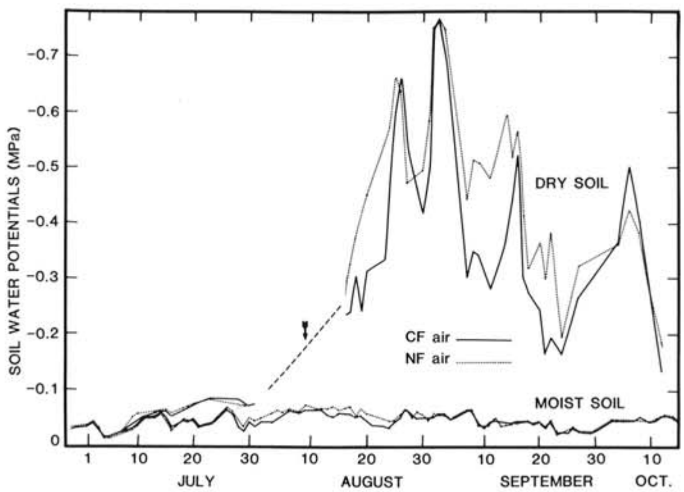

Fig. 2. Soil water potentials for two soil moisture regimes in 1982. A linear interpolation (arrow) is made between 31 July and 17 August for the "dry" soil water potentials since neither tensiometers nor psychrometers measure accurately between −0.08 and −0.15 MPa. Plotted values are the average of daily means at 0.25 and 0.45 m depths in the three replications of each treatment.

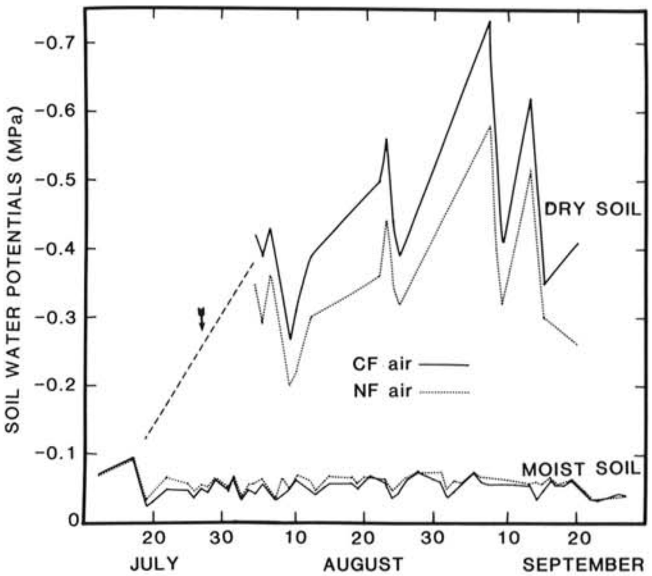

Fig. 3. Soil water potentials for two soil moisture regimes in 1983. A linear interpolation (arrow) is made between 17 July and 4 August for the "dry" soil water potentials since neither tensiometers nor psychrometers measure accurately between −0.08 and −0.15 MPa. Plotted values are the average of daily means at 0.25 and 0.45 m depths in the three replications of each treatment.

the row, a spacing of 0.25 m along the row was accomplished. Water volume was regulated to permit the application of 0.5 cm of water per hour. In the drought series, no more than 0.5 cm of water per day was applied and in the moist series no more than 1.5 cm per day. The amounts added varied depending upon the mean ψ_s at the 0.25 and 0.45 m depths.

The experiment was a completely randomized split plot design and was repeated over 2 yr. Different soil moisture and O_3 treatments were applied to chambers (whole units) with cultivars assigned to rows (subunits) within chambers. Therefore, O_3 and SMS and their interaction were tested against chamber variation (error *a*) while cultivars and cultivar interactions were tested against the within-chamber variation (error *b*). Duncan's multiple range test was used to identify differences between means.

RESULTS

Environmental conditions. June rainfall was above normal for both years. Air temperatures were higher in June through September in 1983 than in 1982. On seven dates in 1983, 7-hr mean values of 0.10 ppm O_3 or higher were measured (Fig. 1). None above 0.08 ppm occurred in 1982 and the 7-hr seasonal means were 10% less in 1982 than in 1983. The average midday (0900 hours to 1600 hours EST) relative humidity values and the maximum and minimum RH values for the 1982 and 1983 field seasons were comparable. Each month from May through September hourly maximum RH values averaged more than 95%. The temporal variability in humidity was also negligible. For example, August of 1982 had 23% of normal rainfall, but the mean RH for August was only 5% lower than for June, July, and September and only 4% less than in August 1983, a month with normal rainfall.

The total irrigation water applied combined with that received from rainfall from May through September was equivalent for the CF and NF air treatments in both years (Table 1). During the 5-mo period, the dry soil series received only half the amount of water received in the moist series; i.e., about 80% of normal rainfall compared to about 160% of normal rainfall for the moist series. No irrigation was needed in May and June. During July, only the moist series was irrigated. In August and September, when water demand by the crop was greatest, plots in the moist series actually received the equivalent of about twice normal rainfall.

The degree of water stress attained in the two regimes is best characterized by ψ_s values over time for the CF and NF air treatments in 1982 and 1983 (Figs. 2 and 3). These data were obtained by averaging on a daily basis the ψ_s of depths of 0.25 and 0.45 m for each treatment. In the dry regime in 1983, ψ_s values were somewhat more negative (lower water potential) in CF air than NF air. The reverse was true for much of the time in 1982. Although for several days the ψ_s values in the dry regime were either too high to be monitored with psychrometers or too low to measure with tensiometers, we interpolated a linear relation; that is, from a July date when tensiometer values were less than −0.08 MPa to the time in August when psychrometry data were available. The ψ_s in the moist series was relatively stable over time due to daily irrigation, if needed, to maintain an average ψ_s of −0.05 MPa for the 0.25 and 0.45 m depths.

TABLE 2. Mean square comparisons for soybean yield and seed size with the 1982 and 1983 data combined

Sources of variation	df	Mean squares[a] Yield (kg/ha) (×1,000)	 Seed size (g/100 seeds)
Year (Yr)	1	29,892***	90.5***
Replication/Yr	4	368	1.0
Ozone (O_3)	1	7,278**	8.3**
Soil moisture (SMS)	1	6,417**	1.1
O_3 × SMS	1	2,717*	1.2
(Rep × O_3 × SMS)/Yr (error *a*)	12	389	0.5
Cultivar (C)/Yr	2	4,217***	129.5***
O_3 × C/Yr	2	123	1.6
SMS × C/Yr	2	1,000*	3.3*
O_3 × sMS × C/Yr	2	513	0.2
Residual (error *b*)	16	247	0.6
R-square		0.94	0.98
Coefficient of variation (%)		9.6	4.6

[a] Asterisks ***, $P < 0.001$; **, $P < 0.01$; and *, $P < 0.05$.

Duration of SMS as well as magnitude of the ψ_s values may affect crop response. In 1982, the late-maturing cultivar Forrest was exposed to SMS conditions 3 wk longer than Williams. In 1983, Williams was exposed longer than Corsoy, but Corsoy in CF air matured only a few days earlier than Williams.

Foliar injury. Based on leaf injury indices ranging from 0 = no injury to 10 = all leaves with injury, there was less of the characteristic O_3 upper surface leaf injury for plants in the dry-soil regime than in moist soil. However, there was more foliage with general chlorosis, a symptom normally associated with drought, in the dry-soil plots. In CF air of both SMS regimes, there was almost no injury of either kind until the plants were in the R-6 stage of growth. Each year, the earliest-maturing cultivar, Williams in 1982 and Corsoy in 1983, showed leaf injury before the later-maturing cultivar.

The biweekly measurements of total stem length and stem length from the cotyledonary node to the first leaf node with a fully green leaf were made in 1983. These data showed no statistically significant differences in number of injured, or senescent, leaves by late August as related to either the O_3 or SMS treatments for either cultivar or between cultivars. Data are not presented, but the biweekly measurements were made from the R-2 (flowering) to the R-7 (physiologic maturity) stage, a period extending from 2 August to 14 September.

Effects on seed size, numbers, and yield. Both O_3 and SMS reduced bean yields significantly (Table 2). There were highly significant ($P < 0.001$) differences in yield and seed size for O_3, SMS, and for cultivars and years. Yields were reduced by O_3 because of effects on seed size (Table 2); whereas with SMS, the effects were due to a reduction in seed numbers (Table 3). In 1982, soil moisture stress reduced yields of Williams in CF air by 13% (Table 4). With Forrest, however, there was sufficient cold injury in the no-stress (CF air-no SMS) treatment to reduce yields below that for the two single-stress treatments.

The cold injury was first observed 24 September. At that time, Forrest was in the R-6 stage but Williams was mature. Beginning 18 September, there were 4 days with air temperatures averaging about 15 C followed by a low of 6 C. It appears that this drop in temperature caused chlorosis to develop in young leaves of plants grown in the well-watered plots with CF air. Since there was very

TABLE 3. The effects of soil moisture stress (SMS) and O_3 on soybean seed and pod numbers, plant height, and stomatal conductance of leaves, 1982[v]

Stress treatment	Per-plant means Seed numbers[w]	 Pod numbers[x]	 Height at harvest (cm)[x]	Leaf stomatal conductance[y] (cm sec^{-1})
No stress	142 a	68 a	150 a	1.8 a
SMS	132 a	61 a	145 a	1.5 b
O_3	144 a	63 a	146 a	1.5 b
SMS and O_3	100 b	41 b	127 a	0.7 c
SEM[z] =	5.4	5.3	3.1	0.09

[v] Mean separation in columns by Duncan's multiple range test, $P = 0.05$; data are combined for the cultivars Williams and Forrest.

[w] Data are total harvest per plot and three plots.

[x] Data are means for eight plants per plot and three plots.

[y] Total conductance of young fully expanded leaves near the top of the plant canopy for each treatment, readings were made between 0800 hours and 1000 hours EST on one leaf of each of six plants on six dates between 23 July and 30 August.

[z] Standard error of the mean.

little, if any, chlorosis in other treatments, apparently the exposure to SMS or to O_3 stress hardened the plants sufficiently to avoid the cold injury. At the time, Williams was approaching harvest maturity so the low temperatures had no effect on it.

With Corsoy, SMS without O_3 stress had no measurable effect on yield whereas Williams in 1983 showed a 15% decrease (Table 4). Ozone reduced yields of Williams 13% in 1983 and an indicated 7% in 1982. In 1983, O_3 without SMS also reduced Corsoy yields by an indicated 7%, but neither of the latter two values were statistically significant.

The combined 1982 and 1983 yield data showed a significant interaction involving O_3 and SMS (Table 2). With the $O_3 \times$ SMS interaction as well as SMS alone, the effects on yield were due to a reduction in seed numbers and not seed size (Table 3). In 1982, numbers of seeds were reduced 30% and numbers of pods were reduced 40% compared to values for the no-stress treatments. Plant height was not reduced significantly by either stress alone or the combined stresses (Table 3).

The predicted yield response for Corsoy, plotted as a function of increasing O_3 concentrations at the two levels of soil moisture, revealed two interactions between these stresses (Fig. 4). At about 0.07 ppm O_3, 7 hr/day for 63 days, the SMS and no-SMS lines intersected. The impact of a combination of higher O_3 concentrations and moderate SMS on yield was less severe than a combination of high O_3 concentration and no SMS. By contrast, the combination of lower O_3 concentrations (ambient range) and moderate SMS at the 0.25 to 0.45 m depth was more severe on yield than when combined with no SMS.

Effects on leaf parameters. Leaf size is reported to be a sensitive indicator of SMS (4). Data from the 1982 season show that leaf size and weight were adversely affected by the combined soil and atmospheric stresses (Table 5). Plants at the flowering, or R-2 stage by Fehr's classification (7), had 26% less leaf area and 18% less weight in plots with both stresses than in unstressed control plots. When pods contained full-size beans (R-6 stage), SMS reduced leaf size in both NF and CF air, 28% when stresses were combined versus 20% with SMS alone (CF air). During the pod-filling or R-5 stage, leaf area and weights were intermediate as expected (Table 5).

The stomatal conductance data in Table 3 indicate relatively little effect on stomatal diffusion due to SMS alone at ψ_s that caused substantial stomatal closure in plots with both stresses. The combination of O_3 and SMS was expected to cause more stomatal closure than either stress alone (22). The ψ_l data further support the conclusion that leaves of plants grown with only SMS were not as heavily stressed as those exposed to the combined stresses. In 1982, measurements of midday ψ_l on sunny days during flowering and pod filling (20 July–15 September), averaged −1.3 MPa for plants grown in NF chambers at −0.44 MPa ψ_s compared to $\psi_l = -0.9$ MPa, for those grown in CF air and similar ψ_s at control depths (Fig. 2). Comparable values for O_3-stressed and nonstressed treatments averaged −0.9 and −0.6 MPa, respectively. The lowest ψ_l measured was −1.7 MPa for soybean plants exposed to both stresses. During early morning, the ψ_l values were in the range of −0.3 to −0.6 MPa, being lowest on plants subjected to both stresses.

DISCUSSION

Evidence is presented indicating that exposure of soybeans to ambient levels of O_3 reduced their tolerance to SMS and increased yield losses. For all cultivars, yields for the treatment with the combined stresses were significantly lower ($P = 0.05$) than for treatments with a single stress (Table 4). The yields were also lower in the treatment with both stresses than for the no-stress treatment. Forrest had apparent cold injury in the no-stress treatment which reduced its yields to levels below those for a single stress. The yields were reduced by the combined stresses because of a reduction in numbers of pods and seeds (Table 3). The interaction did not affect seed size (Table 2). There were more than additive reductions in seed and pod numbers, stomatal conductance, leaf size, and leaf

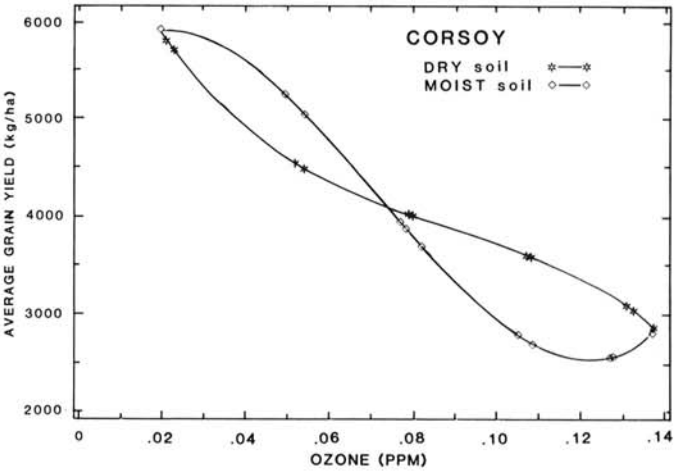

Fig. 4. Predicted response of Corsoy to increasing O_3 concentrations with two soil moisture regimes, 1983. In addition to CF and NF air, there were three replications of each treatment with 0.03, 0.06, and 0.09 ppm O_3 added to NF air. The five levels of O_3 were needed to obtain dose-response functions for use in modeling. The response of Corsoy illustrates best cultivar acclimation to SMS when plants are grown in CF air and the differences in plant yield response in dry and moist soil over a range of O_3 concentrations. Exposures >0.07 ppm O_3 were for 7 hr per day (0900–1600 hours EST) from 23 July to 23 September.

TABLE 4. Yields of soybean cultivars as related to soil moisture stress (SMS) and ambient O_3

	Ozone (ppm, 7 hr/day seasonal means)		Soil moisture (MPa)		Yield (kg/ha) of cultivars[y]			
					1982		1983	
Stress	1982	1983	1982	1983	Williams	Forrest[z]	Williams	Corsoy
No stress	0.016 SE ± 0.0004	0.018 ±0.0005	−0.053 ±0.006	−0.056 ±0.005	5,130 a	4,101 b	7,595 a	5,866 a
SMS	0.017 SE ± 0.0017	0.021 ±0.0011	−0.420 ±0.040	−0.444 ±0.043	4,482 b	5,046 a	6,439 b	5,769 a
O_3	0.046 SE ± 0.0016	0.051 ±0.0016	−0.056 ±0.004	−0.058 −0.007	4,790 ab	4,730 a	6,595 b	5,431 a
SMS and O_3	0.047 SE ± 0.0016	0.053 ±0.0006	−0.466 ±0.031	−0.322 ±0.021	3,193 c	3,528 c	5,755 c	4,413 b

[y] Mean separation in columns by Duncan's multiple range test, $P = 0.05$.

[z] Yield in the no-stress treatment was reduced due to cold injury observed mid-September on this later-maturing cultivar. Plants in the stressed treatments did not show injury apparently due to some hardening. Plants in the no-stress treatments were more succulent. Williams had reached physiological maturity before the cold injury.

weight when the two stresses were combined (Tables 3 and 5). Each year there was a delay in the development of foliar injury on the latest maturing cultivar and less leaf injury in CF than in NF air. Data on foliar injury was of little value in predicting the increased yield loss when both elevated O_3 and SMS were present. This is the first experimental evidence demonstrating that exposure to an air pollutant (O_3) causes plants to lose tolerance to a common natural event (SMS).

The plants seemed to acclimate to SMS in CF air with its reduced O_3 content, but were unable to acclimate in NF air with its elevated O_3 levels. According to Kramer (18) acclimation to drought occurs more readily if SMS develops slowly and a certain limited range of ψ_l is not exceeded. This allows for osmotic adjustment and maintenance of cell turgor so that photosynthetic activity and translocation are not impaired. Osmotic adjustment in cotton leaves was recently documented following exposure to several cycles of alternating low and high ψ_s (1,2). The ψ_l required to close stomata became progressively more negative with each cycle. The adaptation response was attributed to increased cellular solutes due to a starch-induced decrease in cellular volume. Acclimation to SMS has also been observed in other species (18,19,24). Two reports (5,32), however, suggest that soybean plants may not acclimate to drought stress, but the experimental conditions for these studies were very different from those in our investigations. Plants were grown in 10-cm-diameter pots in one of the studies (5). Another experiment (32) involved field-grown plants, but drought stress developed very rapidly and was more severe than in the current experiments. Soybean and sorghum (*Sorghum vulgare* Pers.) cultivar differences in capacities for osmotic adjustment were reported recently (18). Plants of cultivars Williams, Forrest, and Corsoy varied in capacity to adjust to SMS in CF air. Corsoy appeared to acclimate very well to SMS in CF air as shown by Fig. 4. The apparent cold injury in the no-stress Forrest plots tended to prevent a comparison with the other cultivars. Williams was the most sensitive of the cultivars to SMS in both CF and NF air (Table 4). With Williams, the effects of the combined stresses on yield were more than additive in 1982 but not in 1983.

If the present study had not included a CF air control treatment for comparison, the results would have led to interpretations similar to those reported by researchers concerned only with the effects of SMS on soybeans; that is, insufficient water, especially during the flowering and pod-filling stages limits the yields of soybeans (3,6,21,28). Our data indicate, at least under soil conditions similar to those in our study, that much of the soybean yield loss attributed to only SMS is due to an interaction involving SMS and ambient levels of O_3. Investigations conducted under field situations that permit plant utilization of subsoil water by deep-rooted plants may be required to show the interaction between moderate SMS and O_3 stress. Pot-grown plants with severely restricted root volumes may become stressed too rapidly, thus precluding the adaptive response (25).

TABLE 5. Effects of soil moisture stress (SMS) and O_3 on leaf area and dry weight of soybean leaves at different growth stages, 1982[y]

	Leaf area			Leaf dry weight		
Stress	R-2 (flowering) (cm^2)	R-5 (pod filling) (cm^2)	R-6 (pods full) (cm^2)	R-2 (flowering) (mg)	R-5 (pod filling) (mg)	R-6 (pods full) (mg)
None	89 a	78 a	72 a	276 a	308 a	393 a
SMS	82 a	64 b	58 b	270 a	268 b	313 b
O_3	84 a	78 a	76 a	271 a	330 a	384 a
SMS and O_3	66 b	53 c	52 b	226 b	229 c	261 b
SEM[z]	2.6	2.5	3.4	4.0	12.0	20.3

[y] Fehr's classification of growth stages (7); leaf samples are terminal leaflet from fourth trifoliate from stem tip on each of 10 plants per plot, three plots; data from cultivars Williams and Forrest were combined. Mean separation in columns by Duncan's multiple range test, $P = 0.05$.

[z] Standard error of the mean.

Acclimation to SMS in the field may involve root penetration to greater depths permitting utilization of stored ground water as the moisture near the surface becomes inadequate. Characteristically, plants under SMS develop larger root-shoot ratios (18,19,24). This may be due to greater impact of SMS on top growth than on roots, but it may also be due to increase in root growth (27). Soil moisture stress alters the balance of hormones that retard or promote root and shoot growth (19). Abscisic acid levels tend to increase rapidly while cytokinin activity decreases.

Since 1971, there have been reports that exposure to elevated concentrations of O_3 reduces root biomass of soybeans and radish (26,30). However, the combined effects of SMS and O_3 on root growth, especially when plants are grown in the field, are not known. No doubt these effects will depend on the severity of each stress in the combination. In our experiment, the root-shoot ratios increased in response to SMS in both the CF and NF air treatments (H. E. Heggestad, *unpublished*).

There is reason to believe that the plants utilized water stored at depths of more than 1 m as well as water in the primary root zone supplied by rainfall or irrigation during the growing season. In 1983, as determined by soil cores taken just prior to harvest, plants in all plots produced roots penetrating to a depth of about 1.4 m, the approximate location of the water table (H. E. Heggestad, *unpublished*). Soybean roots can penetrate some soils to a depth of 2 m or more (17).

When exposed to the single stresses separately, either moderate SMS or ambient O_3 exposure levels, soybean yields, and the other plant parameters measured were similar to those for plants with no stress; i.e., the CF-moist soil plots. When both stresses were given, yield losses averaged 25%. Seasonal mean 7 hr/day O_3 concentrations in United States soybean production areas are estimated to range from 0.04 to 0.07 ppm, being similar to those at our site (10). Consequently, the somewhat reduced impact of SMS on bean yield when seasonal mean 7 hr/day O_3 concentrations exceeded 0.08 ppm as in Fig. 4 is only of academic interest.

Ozone and SMS may have similar effects on other plant species. For example, there is some evidence that SMS is a contributing factor to the decline of forest species in the northeastern United States and in Germany (15,16). Johnson and Siccama (16) state "...we believe that the evidence regarding a triggering effect of drought is substantiated by our data and those of others, but we do not know whether drought is sufficient to cause the dieback and decline or whether an additional stress from pollution is involved." Ozone was not considered to be a contributing cause to the decline of red spruce (*Picea rubens*) in the northeastern United States, since preliminary experiments with seedlings showed red spruce to be relatively tolerant to O_3 (15). The seedling response to O_3 could be different, however, than that of the more mature trees showing dieback and decline symptoms. We believe that the response to SMS or drought of native species as well as agricultural crops is conditioned not only to exposure to elevated O_3 in ambient air but also by exposure to combinations of phytotoxic air pollutants.

Some concern has been expressed that the field experiments yielded results that were very different from those of greenhouse experiments with O_3 and drought (11,23). According to Treshow and Pack (31), greenhouse results are rarely supported by field observations so far as water relations and fluoride damage are concerned. They report that "... extensive field observations have consistently shown that plants grown under neglected, unfavorable, or arid conditions are the most severely injured by fluoride." On the other hand, controlled greenhouse experiments by others (34) have tended to show that turgid, succulent plants grown under optimum moisture and fertility conditions were the most sensitive to fluoride.

More research under field conditions is clearly needed to assess for a range of plant species, cultivars, and soil conditions the impacts of stress interactions caused by combinations of toxic air pollutants and natural events such as SMS. In research involving SMS, the energy status of the water, especially in the primary root zone, should be monitored during the flowering and fruiting season and related to leaf water potentials.

LITERATURE CITED

1. Ackerson, R. C. 1981. Osmoregulation in cotton response to water stress. II. Leaf carbohydrate status in relation to osmotic adjustment. Plant Physiol. 67:489-493.
2. Ackerson, R. C., and Hebert, R. R. 1981. Osmoregulation in cotton in response to water stress. I. Alterations in photosynthesis, leaf conductance, translocation and ultrastructure. Plant Physiol. 67:484-488.
3. Ashley, D. A., and Ethridge, W. J. 1978. Irrigation effects on vegetative and reproductive development on three soybean cultivars. Agron. J. 70:467-471.
4. Boyer, J. S. 1970. Leaf enlargement and metabolic rates in corn, soybean, and sunflower at various leaf water potentials. Plant Physiol. 46:233-235.
5. Clark, J. A., and Levitt, J. 1956. The basis of drought resistance in the soybean plant. Physiol. Plant. 9:598-606.
6. Doss, B. D., Pearson, R. W., and Rogers, H. T. 1974. Effect of soil water stress at various growth stages in soybean yield. Agron. J. 66:297-299.
7. Fehr, W. R., Caviness, C. E., Burmood, D. T., and Pennington, J. S. 1971. Stage of development descriptions for soybeans, *Glycine max* (L.) Merrill. Crop Sci. 11:929-931.
8. Heagle, A. S., Body, D. E., and Heck, W. W. 1973. An open-top field chamber to assess the impact of air pollution on plants. J. Environ. Qual. 2:365-368.
9. Heck, W. W. 1968. Factors affecting the response of plants to oxidant air pollutants. Annu. Rev. Phytopathol. 6:165-188.
10. Heck, W. W., Adams, R. M., Cure, W. W., Heagle, A. S., Heggestad, H. E., Kohut, R. J., Kress, L. W., Rawlings, J. O., and Taylor, O. C. 1983. A reassessment of crop loss from ozone. Environ. Sci. Technol. 17:572A-581A.
11. Heck, W. W., Taylor, O. C., Adams, R. M., Bingham, G., Miller, J., Preston, E., and Weinstein, L. 1982. Assessment of crop loss from ozone. J. Air Pollut. Control Assoc. 32:353-361.
12. Heggestad, H. E. 1980. Field assessment of air pollution impacts on growth and productivity of crop species. Paper 80-26-1. Preprint 1980. Annu. Meeting Air Pollut. Control Assoc. Montreal, Quebec, Canada. 14 pp.
13. Heggestad, H. E., Heagle, A. S., Bennett, J. H., and Koch, E. J. 1980. The effects of photochemical oxidants on the yield of snap beans. Atmos. Environ. 14:317-326.
14. Heggestad, H. E., and Middleton, J. T. 1959. Ozone in high concentrations as cause of tobacco leaf injury. Science 129:208-210.
15. Johnson, A. H. 1983. Red spruce decline in the northeastern U.S.: Hypothesis regarding the role of acid rain. J. Air Pollut. Control Assoc. 33:1049-1054.
16. Johnson, A. H., and Siccama, T. G. 1983. Acid deposition and forest decline. Environ. Sci. Technol. 17:294A-305A.
17. Kaspar, T. C., Stanley, C. D., and Taylor, H. M. 1978. Soybean root growth during the reproductive stages of development. Agron. J. 70:1105-1107.
18. Kramer, P. J. 1983. Water relations of plants. Academic Press, New York. 489 pp.
19. Levitt, J. 1980. Responses of Plants to Environmental Stresses. Vol. II. Water, Radiation, Salt, and Other Stresses. Academic Press, New York. 607 pp.
20. Menser, H. A., and Street, O. E. 1962. Effects of air pollution, nitrogen level, supplemental irrigation and plant spacing on weather fleck and leaf losses in Maryland tobacco. Tob. Sci. 6:165-169.
21. Momen, N. N., Carlson, R. E., Shaw, R. H., and Arjmand, O. 1979. Moisture stress effects on the yield components of two soybean cultivars. Agron. J. 71:86-90.
22. Olszyk, D. M., and Tibbits, T. W. 1981. Stomatal response and leaf injury of *Pisum sativum* L. with SO_2 and O_3 exposures. II. Influence of moisture stress and time of exposure. Plant Physiol. 67:545-549.
23. Ormrod, D. P. 1978. Pollution in Horticulture. Elsevier, Amsterdam. 260 pp.
24. Paleg, L. G., and Aspinall, D. 1981. The Physiology and Biochemistry of Drought Resistance in Plants. Academic Press, New York. 492 pp.
25. Radin, J. W. 1983. Physiological consequences of cellular water deficits: Osmostic adjustment. Pages 267-296 in: Limitations to Efficient Water Use in Crop Production. H. M. Taylor, W. R. Jordon, and T. R. Sinclair, eds. American Society of Agronomy, Madison, WI.
26. Reinert, R. A., Tingey, D. T., and Koons, C. E. 1971. The early growth of soybeans as influenced by ozone stress. (Abstr.) Agron. Abstr. 1971: 148 Ann. Meet. of ASA, CSA and SSSA.
27. Sharp, R. E., and Davies, W. J. 1979. Solute regulation and growth of roots and shoots of maize plants. Planta 147:43-49.
28. Sionit, N., and Kramer, P. J. 1977. Effect of water stress during different stages of growth of soybean. Agron. J. 69:274-278.
29. Taylor, G. S., DeRoo, H. G., and Waggoner, P. E. 1960. Moisture and fleck of tobacco. Tob. Sci. 4:62-68.
30. Tingey, D. T., Heck, W. W., and Reinert, R. A. 1971. Effect of low concentrations of ozone and sulfur dioxide on foliage, growth and yield of radish. J. Am. Soc. Hortic. Sci. 96:369-371.
31. Treshow, M., and Pack, M. R. 1970. Fluoride. Page D5 in: Recognition of Air Pollution Injury to Vegetation: A Pictorial Atlas. J. S. Jacobson and A. C. Hill, eds. Air Pollution Control Association, Pittsburgh, PA.
32. Turner, N. C., Begg, J. E., Rawson, H. M., English, S. D., and Hern, A. B. 1978. Agronomic and physiological responses of soybean and sorghum crops to water deficits. III. Components of leaf water potential, leaf conductance, $^{14}CO_2$ photosynthesis, and adaptation to water deficits. Aust. J. Plant Physiol. 5:179-194.
33. Walker, E. K., and Vickery, L. S. 1961. Influence of sprinkler irrigation on the incidence of weather fleck on flue-cured tobacco in Ontario. Can. J. Plant Sci. 41:281-287.
34. Zimmerman, P. W., and Hitchcock, A. E. 1956. Susceptibility of plants to hydrofluoric acid and sulfur dioxide gases. Contrib. Boyce Thompson Inst. 18:263-279.